

OCEANIC INSTITUTE & TUNGKANG MARINE LABORATORY COOPERATIVE PROGRAM ON MILKFISH RESEARCH TML ACTIVITIES

PROGRESS REPORT

July, 1986

The Oceanic Institute & Tungkang Marine Laboratory Cooperative Program on Milkfish Research has progressed into the end of its first phase. Implementation of the program has invited the total effort of the milkfish research group and the employment of a high percentage of the milkfish stock in TML. This dedication has resulted in several cases of spawning and some interesting information of the fish.

The major research activities for this program in TML included: the ecosystem study of milkfish broodstock ponds, testosterone feed experiment and the hormone implantation experiments. Due to the nature of the experimental design and the fish, further data collection and the continuation of the program are necessary. Data collected hitherto from the experiments will be presented in this report.

Experiment I: Ecosystem Study of Milkfish Broodstock Ponds Objective:

Broodstock are usually developed from culture ponds in Taiwan due to the difficulties in obtaining wild spawners. Milkfish were reared in ponds for many years until maturation. The characteristics of milkfish broodstock ponds may therefore provide information on requirements of environmental factors for the maturation of the fish. Some physicochemical and biological parameters of the water in the milkfish broodstock ponds at TML are recorded on a continuous basis throughout an entire year.

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## Procedure:

(1) Two 200m<sup>2</sup> concrete ponds (B1 and B2) are each stocked with 30 8-year-old milkfish since November 23, 1984. B1 is bottomed with mud while B2 is remained as concrete. Prescribed diet with a crude protein content of about 43% is fed to the fish every day. Adjustment in feeding quantity is done depending on the appetite of the fish.

Water quality of the two ponds is monitored every 5 days. The parameters considered include temperature, salinity, pH, Secchi disc transparency, dissolved oxygen (DO), chemical oxygen demand (COD), ammonium - nitrogen ( $NH_4^+-N$ ), nitrite nitrogen ( $NO_2^--N$ ), phosphate - phosphorous ( $PO_4^{-3}-P$ ), chlorophyll a and phaeophytin a.

Inspections of the fish were performed on May 24, July 23 and November 20, 1985. The fish became 9-year-old in September. They were employed for the Dosage-Implantation Experiment on March 25, 1986. The fish were moved to other 200m<sup>2</sup> ponds for temporary rearing so that complete clearance of the pond bottom can be done. New mud was distributed evenly on the Jottom of B1 after sunning and tilling of the original bottom mud. The fish were then returned back to the ponds on May 6, 1986. Inspections were performed on the same date, as well as later on June 17, 1986. Water quality are continued to be monitored weekly.

Sex of a mature fish can be determined by abdominal pressure or cannulation. Appearance of milt upon abdominal pressure and ova upon cannulation indicate a male and a female respectively. Fins of the fish are tagged by cuts for identification. Dorsal, anal, pelvic, top and bottom half of the

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caudal fins are cut to indicate DC, AC, PC, TC and BC. Wedgeshaped cuts at the caudal fin indicate TW and BW. Combination of cuts are needed to identify the large number of fish. Cuts are renewed regularly as regeneration of the fins results in confusion of tags occasionally. Record of the maturity of the tagged fish may provide helpful information in the coming future.

The Dosage-Implantation Experiment was started on March 25, 1986. Chclesterol pellets containing two dosages of LHRHa, namely 200ug and 100ug, were implanted intramuscularly into 2 groups of milkfish. Each group possessed 10 fish. Fish in the control group were implanted with nothing or pure cholesterol pellets. Silastic tubings sealed with elastomer containing 250ug  $17\alpha$ - methyltestosterone were implanted simultaneously into all the experimental fish during the first implantation. All the pellets and silastic tubings were produced by Mr. Robin Cheng in Oceanic Institute. Implantation was performed again on May 6, 1986. Inspections of the fish were done on May 6 and June 17, 1986 (Tables I & I ).

(2) Two 200m<sup>2</sup> concrete ponds (III and IV) are each stocked with 30 8 to 10-year-old milkfish since October 31, 1985. A paddle wheel aerator is equipped in pond III since December 12, 1985 for a comparison experiment with pond IV, which is aerated by air blower. The paddle wheel aerator is turned on late afternoon till the next morning every day. Water quality of the ponds is monitored every Thursday. Parameters considered in water quality check and the prescribed diet fed to the fish are the same as that for ponds B1 and B2.

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Due to the demand of fish for the implantation experiments, 8 fish from each pond were removed on March 26, 1986. Four fish for each pond were then moved back on April 21, 1986. The total number of fish in each pond was maintained at 26 thereafter. Inspections of the fish were performed on May 23 and June 20, 1986 (Tables  $\blacksquare$  and V ).

Result:

(1) Data on the parameters of the water quality collected
in B1 and B2 from November 24, 1984 to November 24, 1985 are
shown in Figures 1 - 11. The data collected from November 24,
1985 thereafter are shown in Figures 1a - 11a.

On March 24, 1985, 5 mature females and 15 mature males were identified in B1, while 5 females and 8 males were found in B2. Inspection on July 19, 1985 indicated atresia or immaturity for all fish. On November 11, 1985, only 3 and 1 mature males were found in B1 and B2 respectively.

In the Dosage-Implantation Experiment, fish were implanted and inspected regularly. On May 6, 1986, the cannulated ova of 2 females in B2 was found to be more than 0.6mm in diameter, the fish was moved to a  $19.54m^2$  octagonal concrete pond for spawning. Five males in B2 with abundant or medium amount of running milt upon abdominal pressure were also moved to the spawning pond. Injection of LHRHa to the females induced the spawning of the fish BW on May 15, 1986, producing more than 489,000 eggs (Table  $\mathbb{M}$ ). However, no fertilization occurred. Ova cannulated from the same fish BW on June 17, 1986 were again appropriate for induced spawning. Nevertheless, injection failed to induce spawning of the fish on June 17, 1986. The

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result of the inspections are shown in Tables I and II.

(2) Data on the parameters of the water in ponds III andIV from January 2 to May 29, 1986 are shown in Figures 12 - 22.

Maturity of the fish were inspected on May 23 and June 20, 1986. Fin cut was also performed to identify each fish. Appropriate mature fish were also moved to spawning pond for spawning, as described. Induced spawning of the fish ADC and ADPTW from III were occurred on May 25, 1986. The numbers of eggs collected were 399,600 and 649,600 respectively. No fertilization occurred. On the other hand, induced spawning of the fish TW from IV was failed (Table II).

Altogether 9 mature females, 3 males and 6 females, 8 males were identified in ponds III and IV respectively on May 23, 1986. Only 1 male and 4 males, 1 female were found in III and IV respectively on June 20, 1986. Atresia due to stress may be the reason for this result. Tables III and V show the result of the inspections.

Experiment II: Testosterone Feed to Induce Maturation Objective:

Maturatioon has been induced successfully in grey mullet by the oral administration of  $17^{\alpha}$ -methyltestosterone in OI. Spermatogenesis has also been enhanced in milkfish in a similiar experiment. Based on the result of these experiments performed in OI, an experiment is performed in TML to test the effectiveness of testosterone feed on rematuration of male milkfish that have demonstrated maturity before.

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#### Procedure:

Male 8 to 10-year-old milkfish from previous hormone experiment in 1980 have been stocked in two 8.4m diameter round concrete ponds (R3 and R8) since April 2, 1985. Five males in R3 are fed with testosterone in their diet while four males in R8 are fed with control diet starting April 16, 1985. A fish used for the hormone implantation demonstration during the Milkfish Workshop in 1985 are reared in R8 to increase the stocking rate to 5 fish/pond. The feed is a high protein diet with a CP content of about 44%. The hormone is provided by OI in spirulina powder of which 10 g/kg feed is fed to the experimental fish every day. Inspection for the maturity of the fish is performed regularly.

The fish were moved to  $19.54m^2$  octagonal concrete ponds (T1 and T2) on March 21, 1986 due to the demand of round ponds for the hormone implantation experiments. Inspections were continued thereafter (Table VI ).

## Result:

Data for the inspections of the fish is shown in Table VI. From the data collected, testosterone in the diet of the fish does not seem to be the factor inducing the maturation of the fish. The spawning season of the fish is probably occurred from May to October, as both the control and experimental fish show maturity. No mature fish has been found from November, 1985 till March, 1986. Since the change of ponds on March 21, 1986, only one experimental fish showed maturity on April 24 and June 18, 1986. The new environment may have par-

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ticular negative effect on the maturation of the fish. The effect of the testosterone to the maturation of the male milkfish is therefore still unclear.

Experiment III: Hormone Implantation

### Objective:

Injections of hormone to induce maturation or spawning of milkfish often result in mortality due to stress. Methods of hormone application of which little stress is caused is therefore wanted for the artificial propagation of milkfish. Intramuscular implantation provides a chronic effect of the applied hormone to the fish. Stress caused by handling are therefore reduced.

Maturation and spawnings of milkfish have occurred upon the intramuscular implantation of LHRHa and other hormone in OI. This technology to induce maturation of milkfish is tested in TML. The eligibility of this technique may be determined by the implementation of several experiments.

### Procedure:

(1) Milkfish of two different age groups (4.5 and 9.5 years) were employed for implantation experiment on April 27, 1985. Eight fish from each group were each implanted with a pellet containing 180ug LHRHa and a capsule containing 250ug liquid testosterone. The fish were reared in two 8.4m diameter concrete ponds (R9 and R10) since May 11, 1985. A commerical feed with a CP content of 44% was fed to the fish. Inspections were performed regularly since May 27, 1985.

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The fish became 5 and 10-year-old on September, 1985. The 10-year-old fish were moved to a 19.54m<sup>2</sup> octagonal concrete pond on March 31, 1986. Experiment for the 5-year-old fish was terminated on the same date.

Pellets containing 200ug LHRHa were implanted into the 10-year-old fish on March 31 and April 24, 1986. Silastic tubings containing 250ug  $17\alpha$ -methyltestosterone were also implanted during the first implantation. Inspections were implemented on May 23 and June 18, 1986 (Table VI).

(2) Dosage-Implantation Experiment

Fish from ponds B1 and B2 were employed for this experiment as described. Three groups of 10 fish were implanted with pellets containing 200ug, 100ug or 0ug LHRHa respectively. Inspections of the fish were performed regularly (Tables I and

# (3) Age-Implantation Experiment

Six age groups of milkfish, namely 4, 5, 6, 7, 8 and 8 -10 years, are employed for a comparative experiment to determine the effect of hormone implantation to the maturation of the fish. Each group possesses 10 experimental fish and 6 control. Pellets containing 200ug LHRHa have been implanted into the experimental fish, while cholesterol pellets have been implanted into the control ones. Silastic tubings containing 250ug 1% -methyltestosterone have also been implanted into the experimental fish on March 26 or 27, 1986. The fish are reared in 8.4m diameter round concrete ponds (R8, R3, R4, R9, R5, K10 respectively) and fed with the same diet as the fish in ponds B1, B2, III and IV. Inspections are performed regularly. Appropriate mature fish are moved to octagonal ponds

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for spawning (Tables M - X ).

Result:

(1) Result of the 1985 implantation experiment and data on the continuation of the experiment in 1986 are shown in Table  $\bigvee$ I. Six out of the 8 fish in R10 (9.5 years) and 4 out of the 8 fish in R9 (4.5 years) are determined to be male. Unfortunately 1 female, 2 males and 1 male in R10 and R9 respectively died during the experiment. The weight of the gonad (GW) of the dead fish are found to be small. No appearance of ova has been observed upon cannulation of any of the experimental fish. Mature fish are found only from May to November, which is probably the spawning season of milkfish in Taiwan.

After the change of pond on March 31, 1986, implantation were performed twice to the 10-year-old fish. However, no mature fish are yet to be found. As mentioned before, the new environment at the octagonal ponds may have particular negative effect to the maturation of the fish. Acclimation of the fish to the new environment may improve the condition of the fish in the near future.

(2) The result of the Dosage-Implantation Experiment is shown in Tables I and II. Confusion of tags has occurred during inspections of fish in B1, which results in vague data for several fish.

Apparently, the higher dose of hormone has a slightly stronger effect than the lower dose. Number of mature fish in B2 is abundant. More than 50% of the fish implanted with high dose of hormone become mature, while maturity of the fish implanted with low dose of hormone is slighter. Spawning of the fish BW probably indicates the effect of the high dose of

- 9 -

implantation.

Nevertheless, the fish in Bl did not show the same degree of maturity as those in B2. This situation is the opposite as the situation in 1985 when more mature fish were found in Bl than B2. The new environment developed by the application of new mud to the pond may possibly demand effort of the fish in adaptation. Many other reasons may cause the difference in maturation of the fish in the 2 ponds. Further inspections will be performed to study the effect of dosage, environment and maturation of the fish.

(3) The result of the Age-Implantation Experiment is shown in Tables VII - XIII. Ages of the fish are determined by the number of years the fish being reared in TML since capture as wild fry. For the convenience of record, the ages of all fish advance on September of each year.

No maturity has been found in the 4-year-old fish in R8 (Table VII). The fish are probably too young for maturation as gonadal development has yet to complete. The 5-year-old fish in R3 appear to be affected by the hormone implantation. Four mature males have been identified after 3 implantation. However, the quantity of running milt upon abdominal pressure has found to be petty (Table X ).

The maturity of the 6-year-old fish in R4 seems to be more conspicuous than the 5-year-old ones in R3 (Table X). Ova with an average diameter of 0.529mm have been cannulated from a large female on June 18, 1986. The maturity of the 7year-old fish in R9 appears similiar to those 6-year-old in R3 (Table XI). Ova cannulated from 2 females on May 21, 1986 measured 0.63mm and 0.36mm in diameter. The quantity of running

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milt from 1 male was abundant.

The maturity of the 8-year-old fish in R5 was distinguished. Males with abundant running milt and females with large cannulated ova were found during inspections (TableXII). The female ABC failed to spawn upon injection on LHRHa even though its ova measure 0.78mm in diameter in April, 1986. The control fish DPC also failed to spawn upon implantation of hormone pellet in May, 1986. Finally, the control fish DBW spawned 2 days after the injection of 250ug LHRHa on June 18, 1986. A total of 499,500 eggs were then collected (Table II). However, no fertilization occurred. Implantation did not seem to be the factor inducing the maturation of the fish in R5. Both the control and experimental fish were found to be matured. Age may then be a more important factor in the maturation of the fish.

Abundant running milt from 3 experimental fish and 2 control were obtained during the inspection of the 8-10-yearold fish in R10 on April 24,1986 (Table  $X \blacksquare$  ). The female BW spawned scarce number of eggs upon injection of LHRHa on May 24, 1986. The female BC also spawned 2 days after the same dose of injection on June 18, 1986. Mere 42,700 eggs with no fertilization were then collected (Table  $\blacksquare$  ). The experimental fish seem to mature more readily than the control ones in R10. However, old age may also be an important factor for the maturation.

Table X III summarized the result of the Age-Implantation Experiment. Further inspections will definitely result in more detailed information concerning the effect of hormone implantation.

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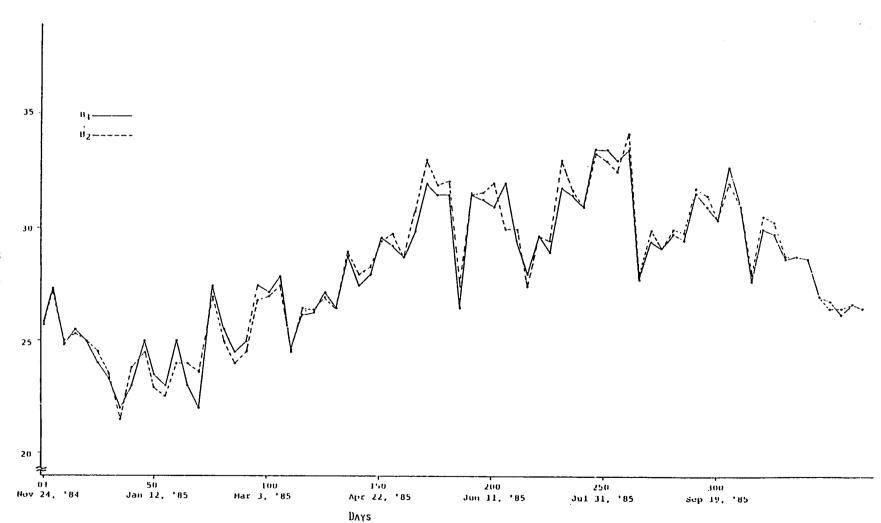


Fig. 1. Temperature of water from ponds B<sub>1</sub> and B<sub>2</sub>.

Темреядтике (<sup>G</sup>C)

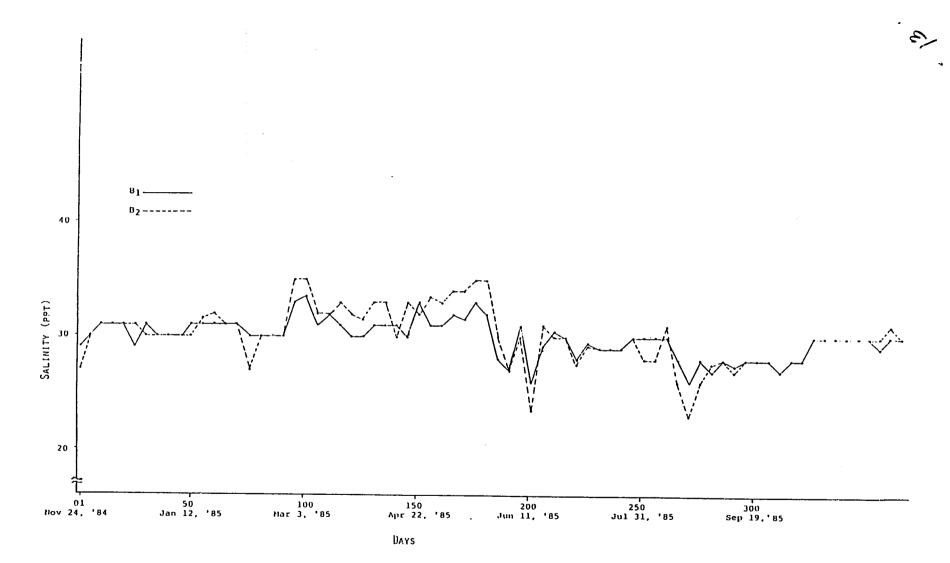


Fig. 2. Salinity of water from ponds  $B_1$  and  $B_2$ .

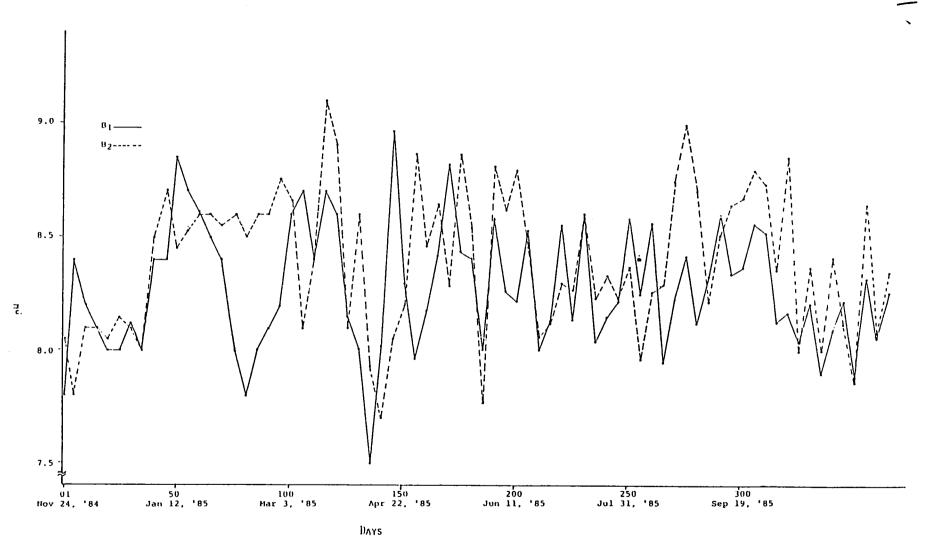


Fig. 3. p[-] of water from ponds  $B_1$  and  $B_2$ .

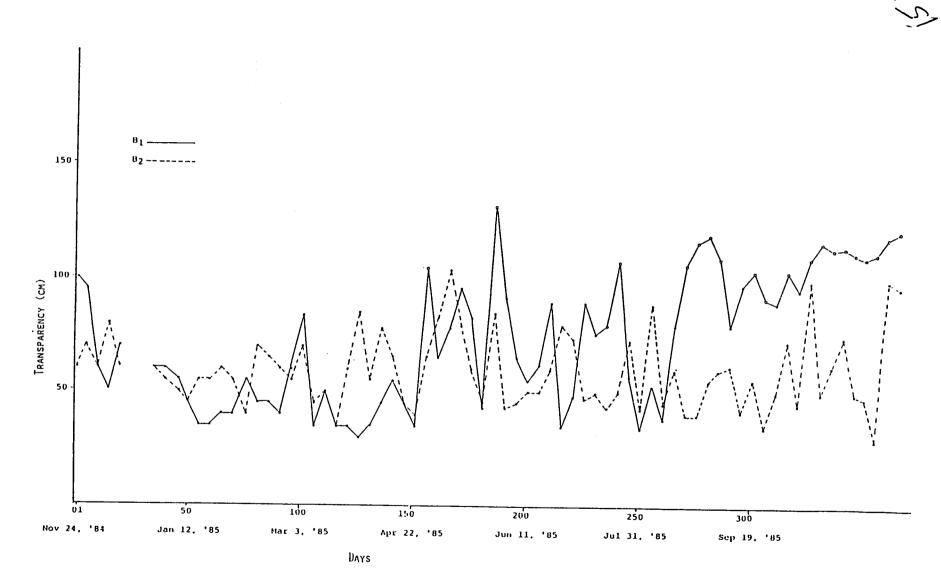


Fig. 4. Seechi disc Transparency of water from ponds  $B_1$  and  $B_2$ .

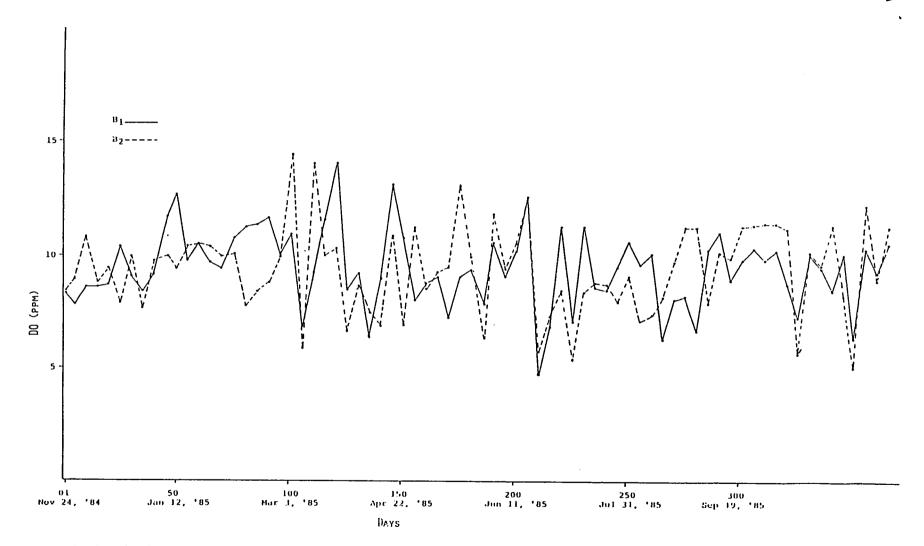


Fig. 5. Dissolved Oxygen in water from ponds B1 and B2.

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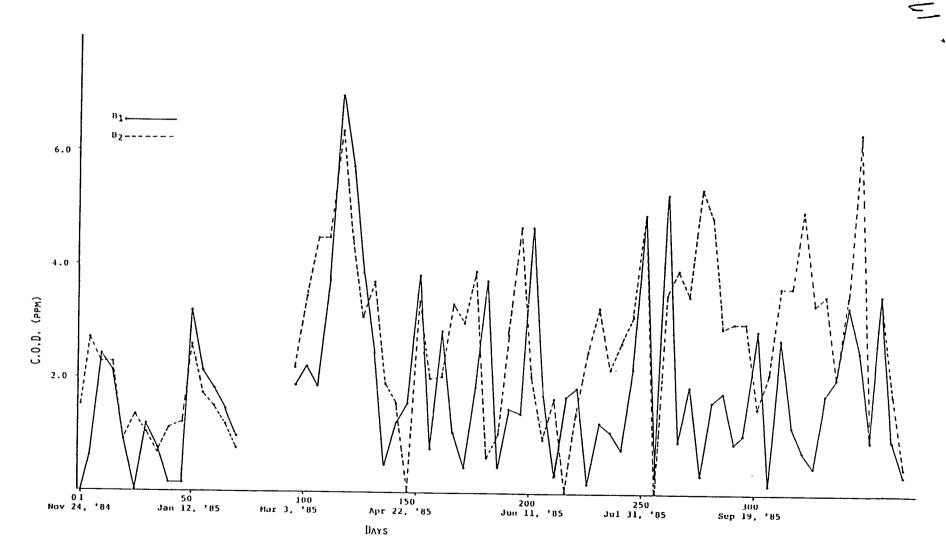


Fig. 6. Chemical Oxygen bemand of water from ponds  $B_1$  and  $B_2$ .

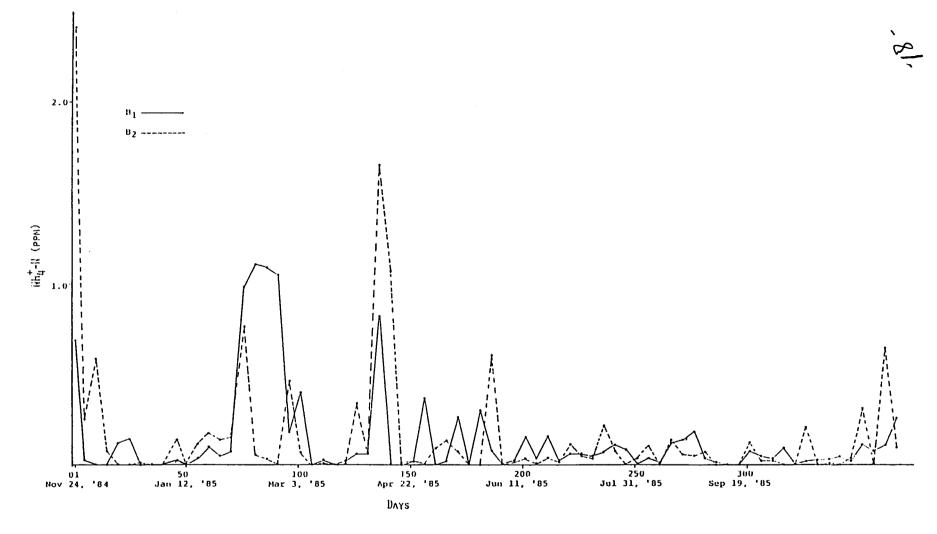


Fig. 7. Ammonium-nitrogen content in water from ponds B1 and B2.

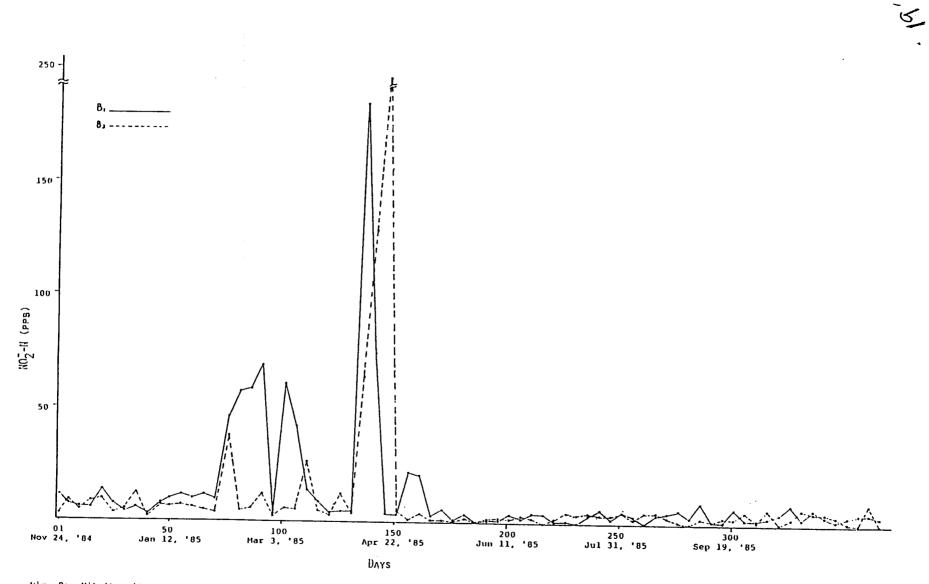


Fig. 8. Nitrite-nitrogen content in water of ponds  $B_1$  and  $B_2$ .

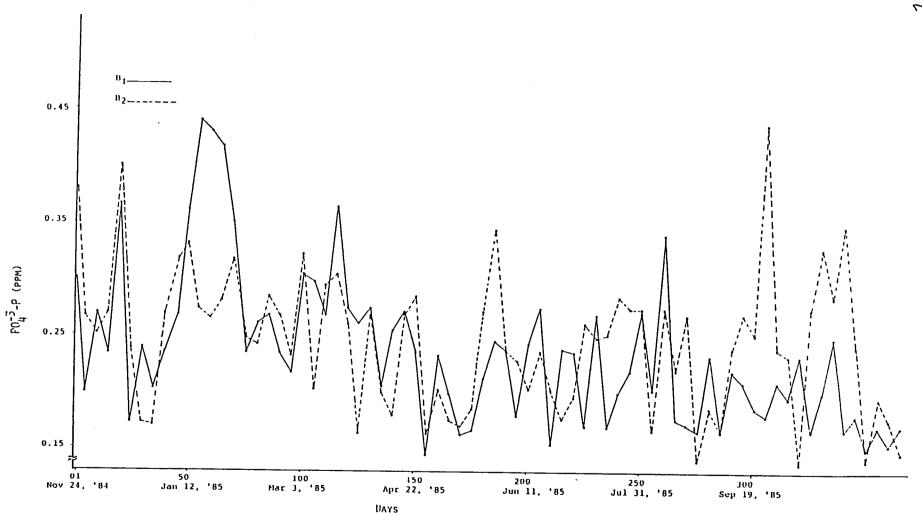


Fig. 9. Phosphate-phosphorous content in water from ponds  $B_1$  and  $B_2$ .

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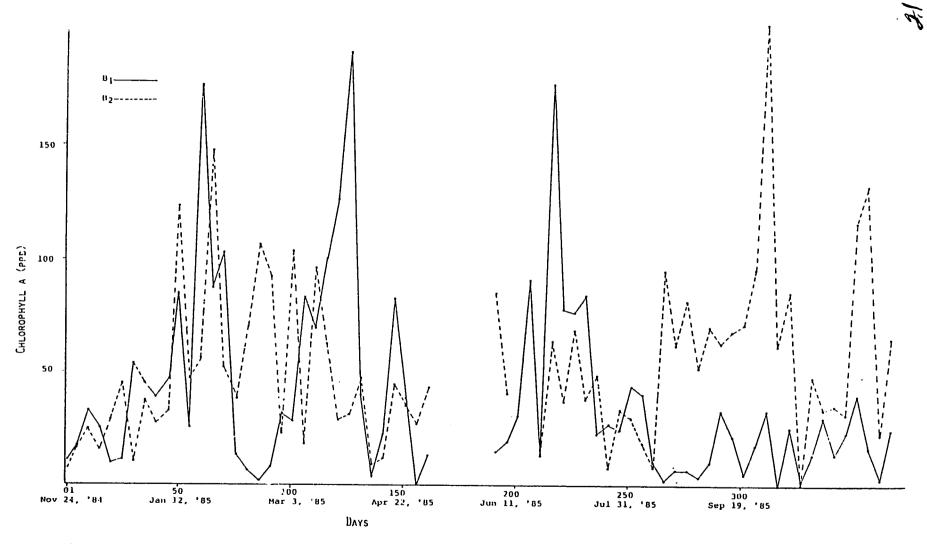


Fig. 10. Chlorophyll a content in water of ponds  ${\rm B}_1$  and  ${\rm B}_2.$ 

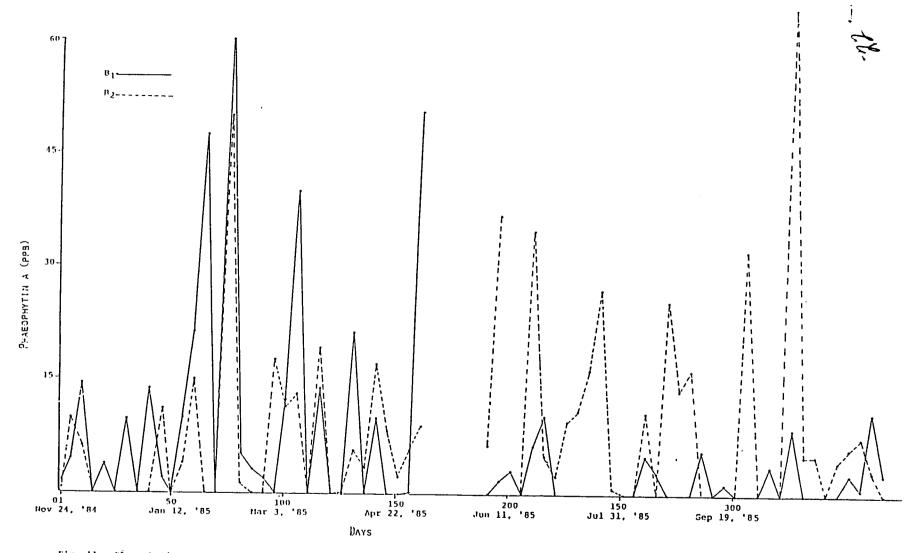
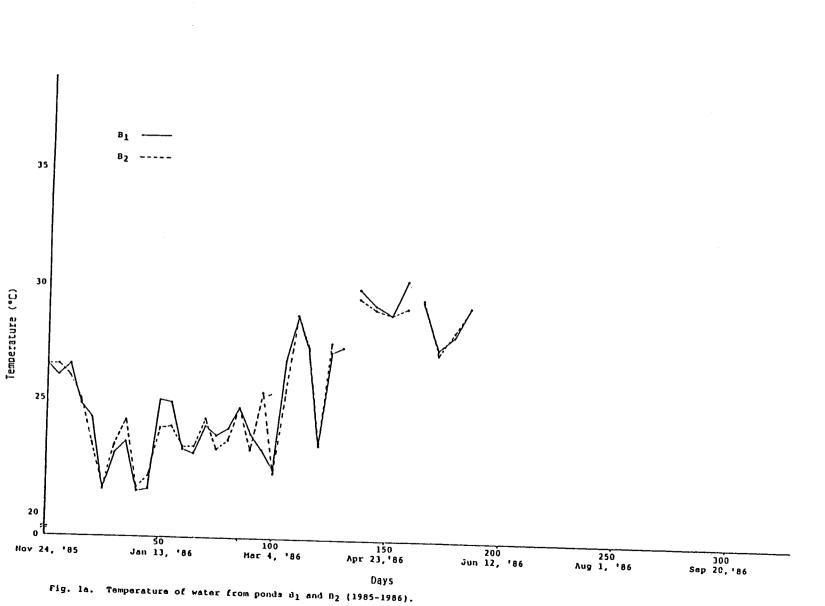


Fig. 11. Phaeophytin a content in water of ponds  $B_1$  and  $B_2$ .



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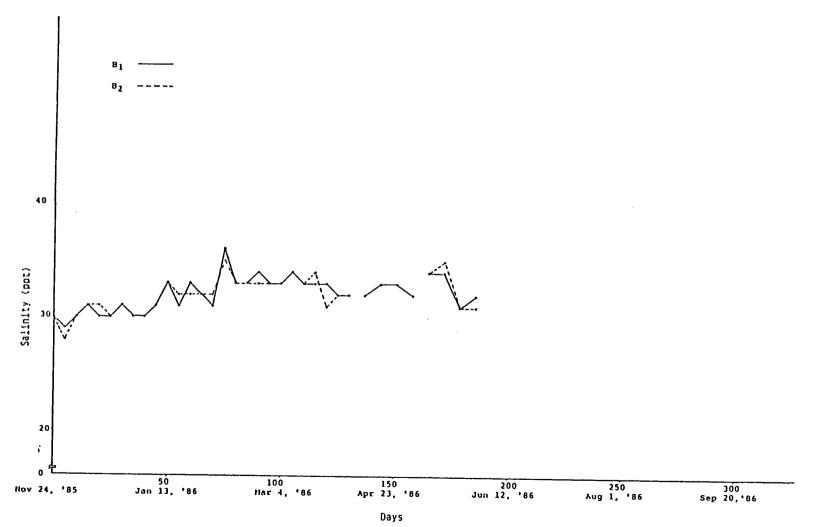
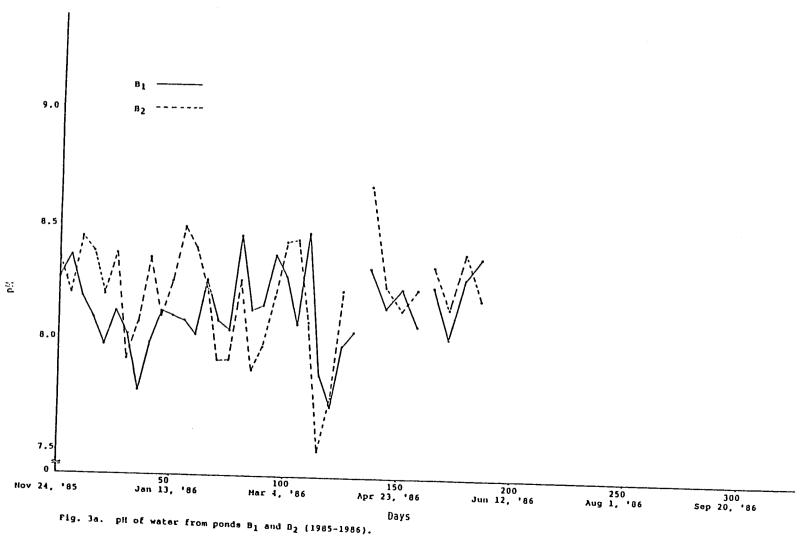


Fig. Za. Salinity of water from ponds  $B_1$  and  $B_2$  (1985-1986).

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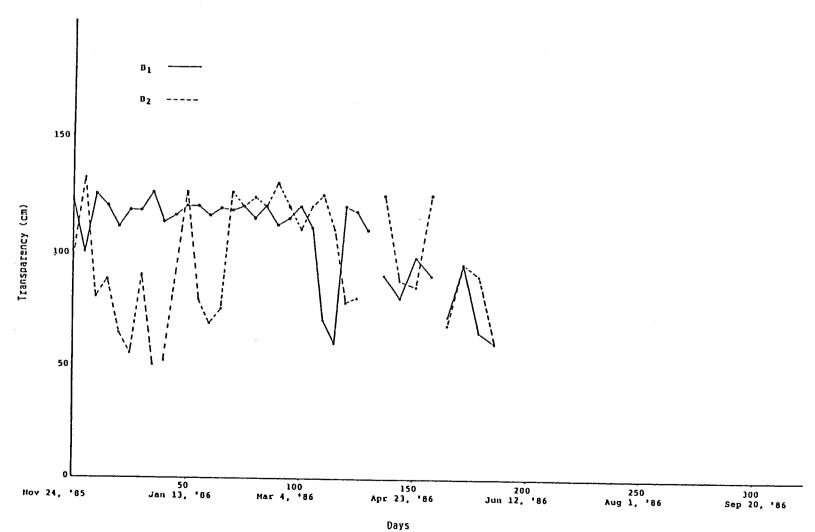


Fig. 4a. Secchi disc Transparency of water in ponds  $B_1$  and  $B_2$  (1985-1986).

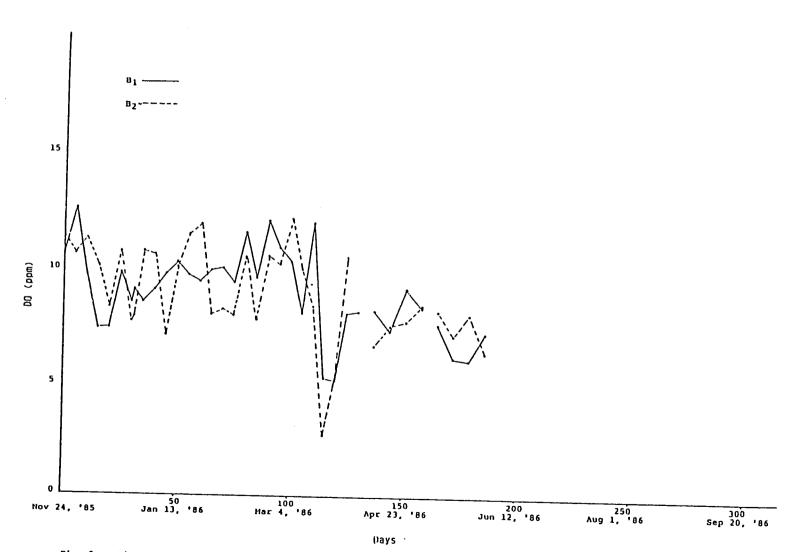


Fig. 5a. Dissolved Oxygen in water from ponds  $B_1$  and  $B_2$  (1985-1986).

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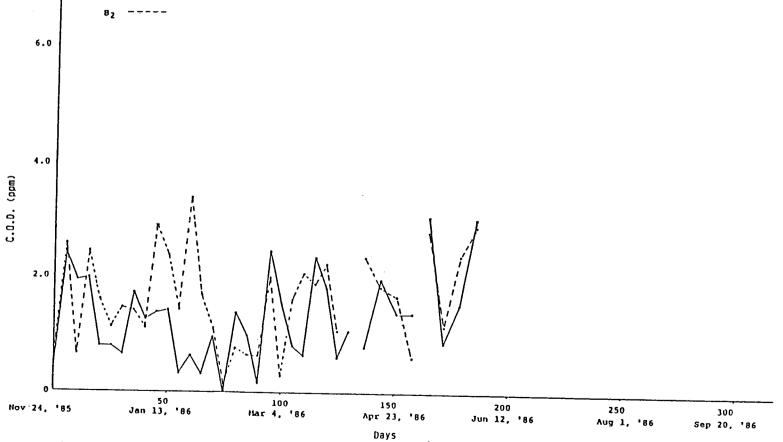
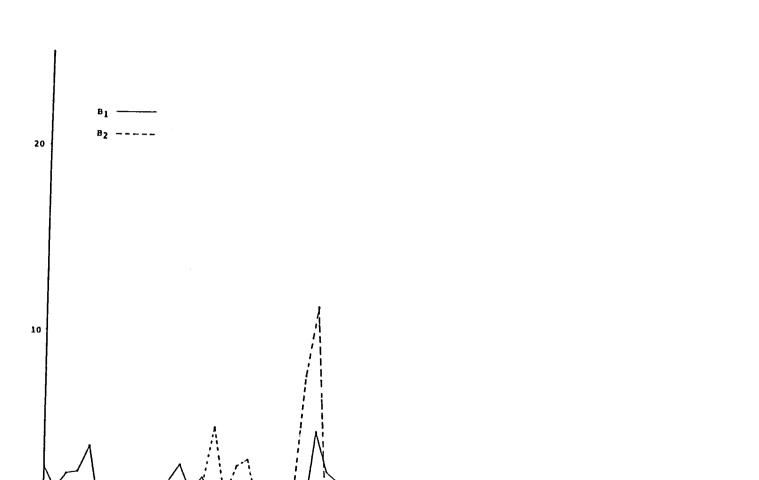


Fig. 6a. Chemical Oxygen Demand of water from ponds  $B_1$  and  $B_2$  (1985-1986).

81



150 Apr 23, '86

Days

200 Jun 12, '86

250 Aug 1, '86 5

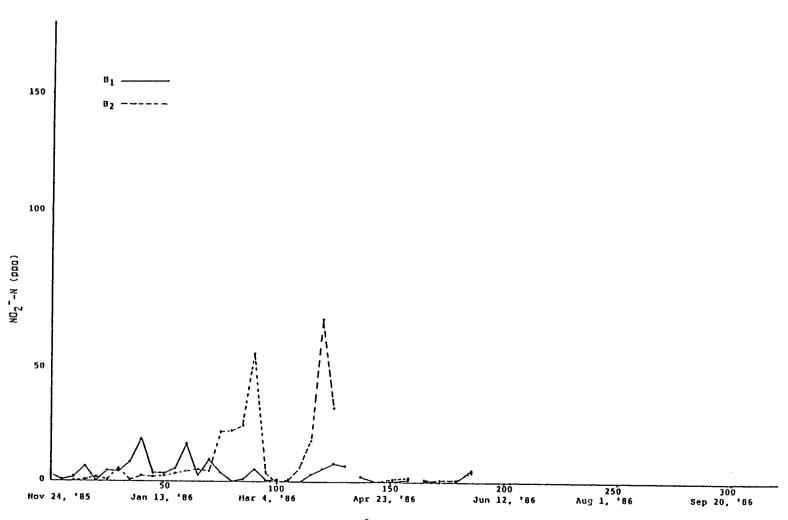
Fig. 7a. Ammonium-nitrogen content in water from ponds B<sub>1</sub> and B<sub>2</sub> (1985-1986).

100 Mar 4, '86

50 Jan 13, \*86

(mod) N-<sup>+</sup><sup>7</sup>HN

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Days

Fig. 8a. Nitrite-nitrogen content in water of ponds  $B_1$  and  $B_2$  (1985-1986).

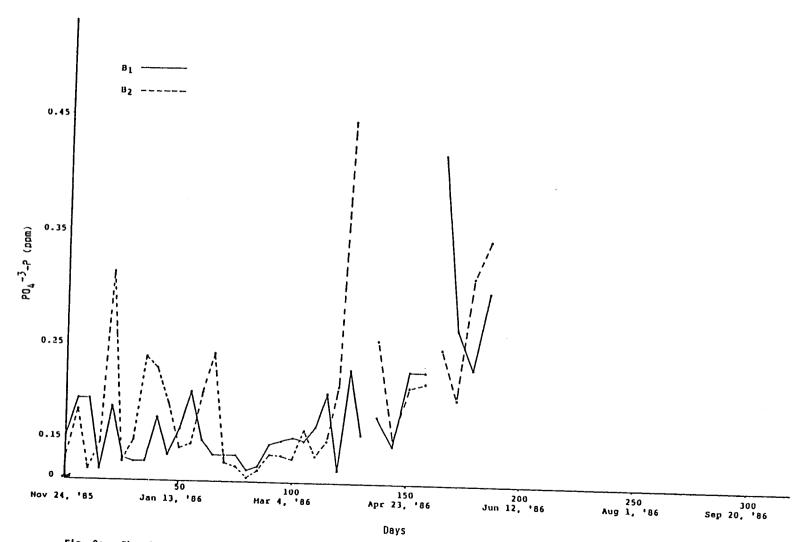


Fig. 9a. Phosphate-phosphorous content in water from ponds  $B_1$  and  $B_2$  (1985-1986).

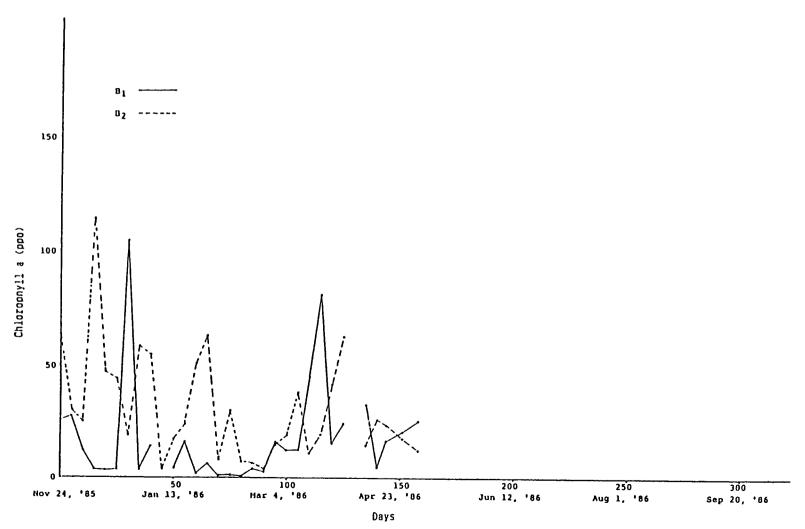


Fig. 10a. Chlorophyll a content in water of ponds B1 and B2 (1985-1986).

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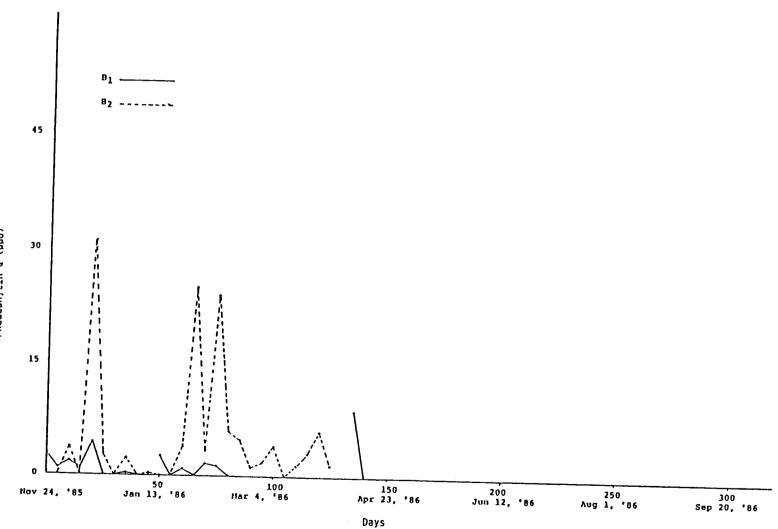
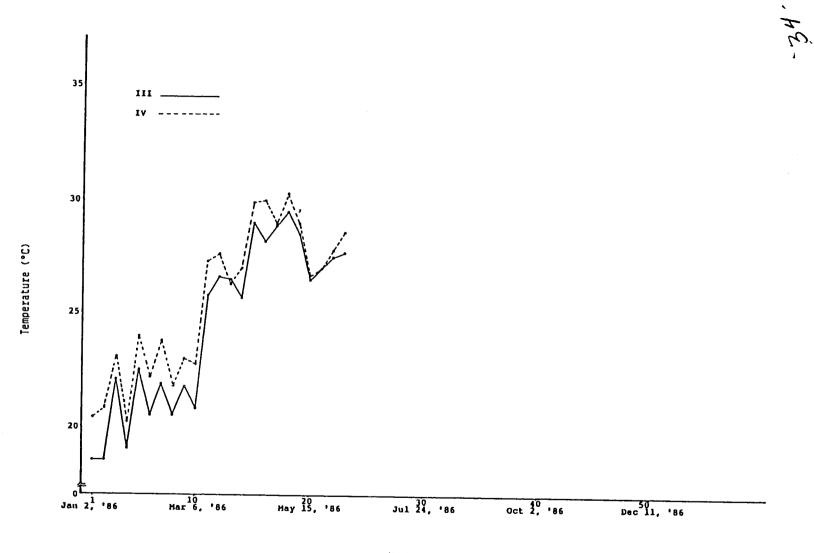


Fig. 11a. Phaeophytin a content in water of ponds  $B_1$  and  $B_2$  (1985-1986).

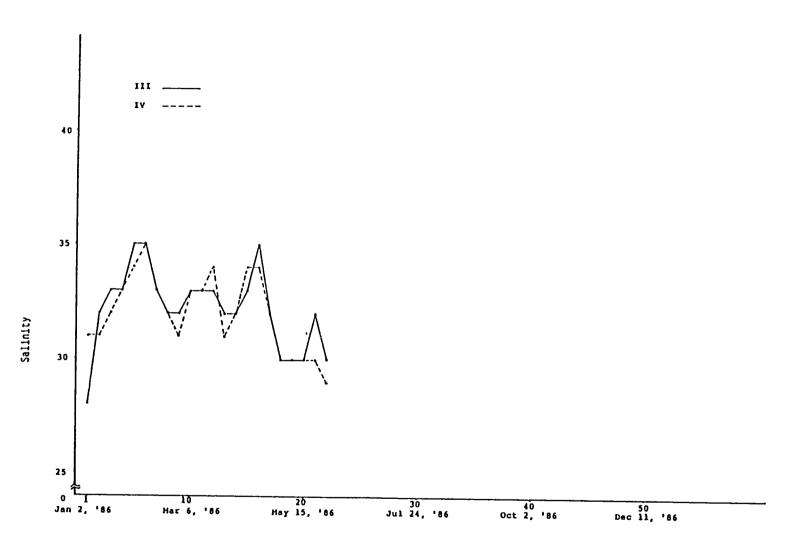
Phacopiytin a (pp0)

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Weeks

Fig. 12. Temperature of water in ponds III and IV.



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Weeks

Fig. 13. Salinity of water in ponds 111 and IV.

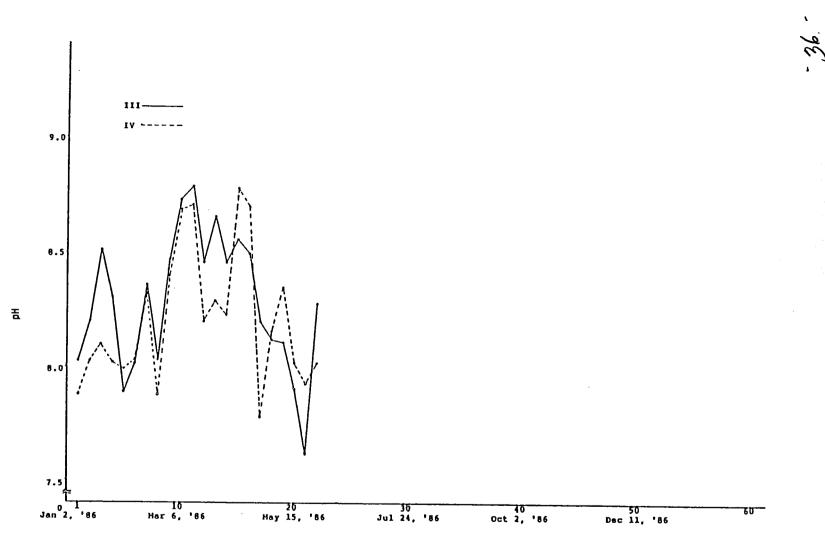
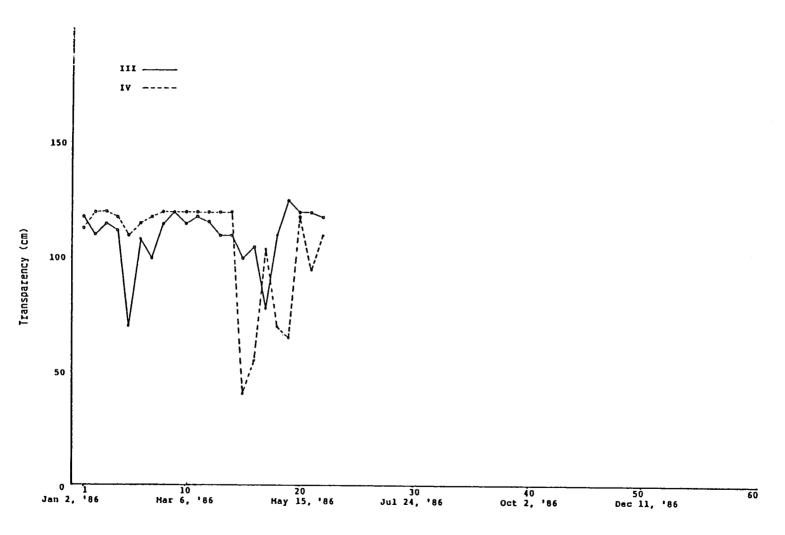


Fig. 14. pH of water in ponds III and IV.



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Fig. 15. Secchi disc transparency of water in ponds III and IV.

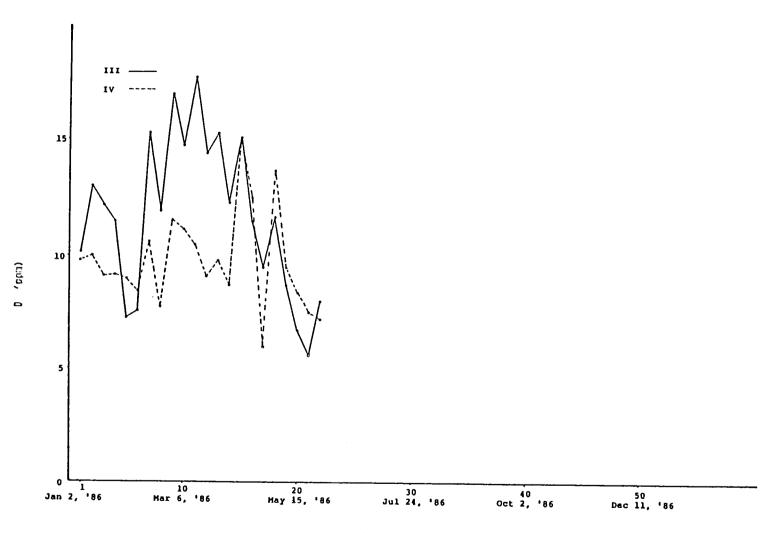
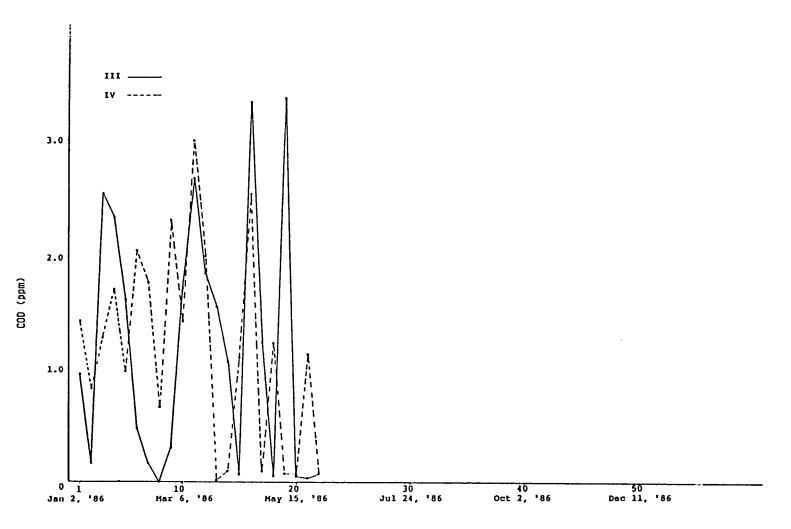


Fig. 16. Dissolved oxygen in water of ponds III and IV.

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Fig. 17. Chemical oxygen demand of water of ponds III and IV.

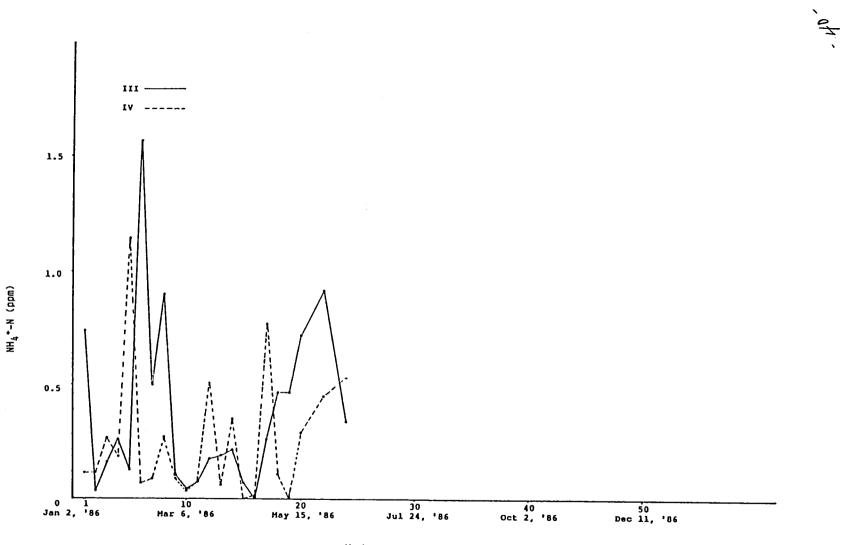


Fig. 18. Ammonium-nitrogen content in water of ponds III and IV.

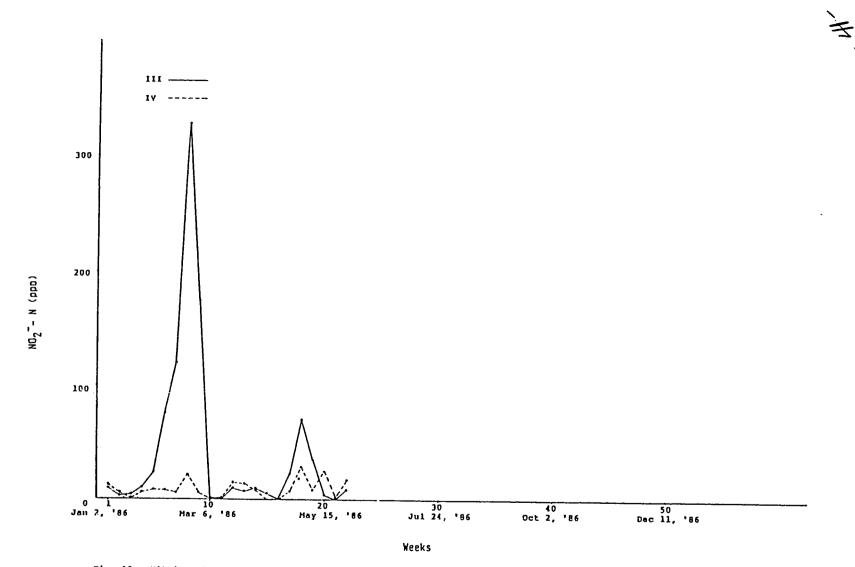


Fig. 19. Nitrite-nitrogen content in water of ponde III and IV.

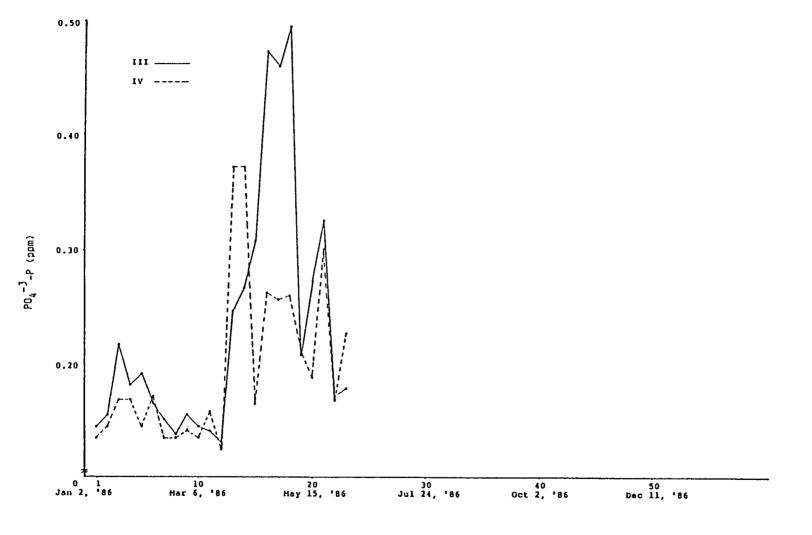


Fig. 20. Phosphate-phosphorous content in water of ponds III and IV.

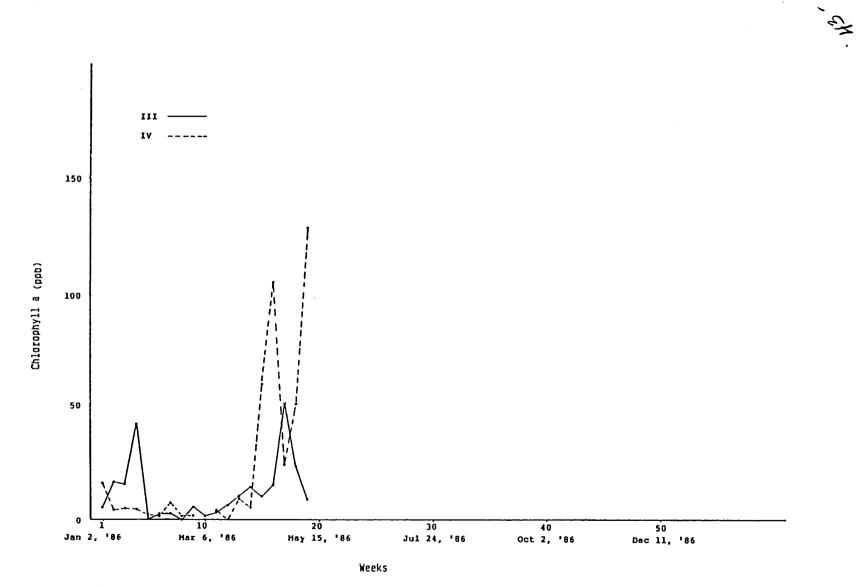
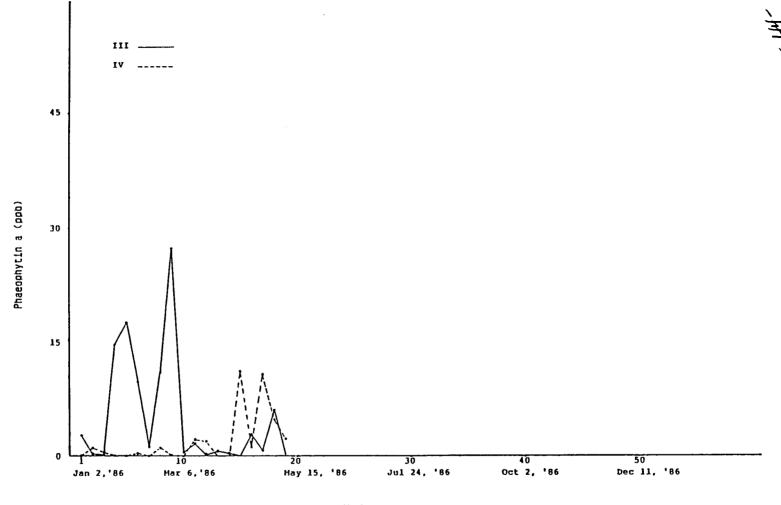


Fig. 21. Chlorophyll a content in water of ponds III and IV.



Weeks

Fig. 22. Phaeophytin a content in water of ponds III and IV.

Fish tag	Ma BW	r 25 FL			Мау б		Jun 17
	(kg)	(cm)	Treatment*	condition	Treatment*	condition	condition
TC	2.82	54.0	200	-	200		<u> </u>
BC	3.08	53.0	200	-	200	-	_
TN	3.16	54.5	200	-	200	-	_
9W	4.02	59.0	<b>2</b> ້00	previt. 0.289	200	+	-
DTC	3.80	58.0	200	-	200	-	+•
DBC	3.90	59.0	200	-	200	-	_
DTW	3.96	62.0	200	previt.	200	-	_
DBW	4.20	59.0	200	-	200	+++	_
PC	3.31	53.5	200	-	200	-	_
DPC	3.74	57.8	200	previt.	200	-	+•
ATC	3.18	56.0	100	-	100	-	_
ABC	3.10	57.0	100	-	100	· •	_
ATW	2.97	54.0	100	-	100	-	<b>+</b> •
ABM	3.60	58.5	100	previt. 0.317	100	+	+
DTC	4.56	61.0	100	-	100	-	-
DBC	3.02	56.5	100	previt.	100	<b>_</b> ·	-
DTW	3.02	53.0	100	-	100	-	-
DBW	2.86	51.5	100	-	100	-	-
PC	2.61	50.5	100	-	100	-	_
DPC	3.18	56.0	100	-	100	***	-
0							
ontro	l witho	ut tag		l previt.		2 +++	1 +•

Table [ Record on inspection and treatment of milkfish in pond 81 (Dosage - Implantation Expt.)

\* ug LHRHa; control fish were implanted with cholesterol pellets.

 milt appear yellowish in colour previt.: previtellogenesis

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Fish	<b></b>		r 25		May	6		Jun 17	,
tag	BW (kg)	FL (cm)	Treat- ment*	condition	Treat- ment*	condition	BW (kg)	FL.	
TC	2.46	52.5	200	-	200				0.380
BC	3.99	53.0	200	-	200	++ .			?
TW	3.48	54.5	200	-	200	-			• ++ ©
BW	6.89	68.0	200	previt.	200	0.635 ●			0.640 3
DTC	3.72	60.7	200	-	200	0.700 \$	2)	. GN	0.718 Dead = 157.40 g)
DBC	2.87	52.5	200	-	200	++ S	• 1	,	- 13/.40 g)
DTW	5.99	68.0	200	-	200	-			-
DBW	2.70	50.0	200	-	200	DPSW			++ 5
PC	3.14	54.8	200	-	200	-			++ (S)
DPC	3.34	57.6	200	Dead (3,	GW = 6.27	5 g)			rr (gr
ATC	2.75	51.0	100	-	100	-			++ ?
ABC	2.72	51.0	100	-	100	-			+
ATW	3.74	57.5	100	-	100	+++ 5			+ 5
ABW	2.63	51.5	100	-	100	-			?
ADTC	3.41	57.0	100	Dead (8,0	GW = 6.35(	)g)			•
ADBC	4.19	59.0	100	-	100	+++ 5			+
ADTW	4.69	63.0	100	-	100	-			-
ADBW	2.50	49.5	100	-	100	-			-
APC	2.67	53.0	100	-	100	+ 9			-
AD9C	3.12	54.0	100	-	100	-			0.532
					200	+++ S DBW			*++ <b>(</b> 3)
LO									
Contro.	l witho	ut tag			9	1 +	2.40	52.0	-
					control	1 +++	2.80	55.4	+
				l missing	without		2.30	52.0	
					tag		2.50	51.0	++ 9
							2.84	54.0	-
							2.94	55.0	-
							3.72	56.0	0.461
							2.40	51.7	-
							2.50	53.2	-
							2.28	50.0	-
							2.38	54.5	

Table IRecord on inspection and treatment of milkfish in pond B2<br/>(Dosage - Implantation Expt.)

\* ug LHRHa; no treatment for control fish.

 Fish spawned; S Fish were moved to spawning pond for spawning previt.: previtellogenesis

Ne

	Fi	.sh				Treat	mei	nt	Ξ	ggs collectio	n
	Origin	Age	Date					Injection or implantation	Date	Est. Number	Diameter (mm)
12;		8	Mar 20 Apr 20	5 .				implantation* 250 ug LHRHa liquid	-		
39:	R10	8-10	Mar 2	7, Αρε	24			implantation (2 $\hat{o}$ )*			-
29;		9	Mar 25 May 11		6			implantation* 250 ug LHRHa liquid	Mar. 15		
5 ô :	82	9	Mar 25		6			implantation (2 3*, 3 8 **)	May 15	489,000	1.130
2 <del>9</del> :	R10	8-10	Mar 27 May 24		24	May	21	implantation*			
33:		8-10		, Apr	24,	Мау 1	21	250 ug LHRHa (1 9) implantation (2 8)* 300 IU HCG	May 25	SCAFCE	-
ι <sub>ġ</sub> :	R10	8-10	Mar 27 Jun 18		24,	May 2	21	implantation* 250 ug GHRHa (China)	· ··		
4 8 :	R10	8-10		, Apr	24,	Мау 2	21	implantation (3 3)* 350 IU HCG (3 3)	Jun 21	42,700	1.185
17: 18:		8 8	May 21 Mar 26		23			implantation* implantation*	-	-	-
12:	32	9	Mar 25 Jun 17		6			implantation*			
5 6. :	82	9	Mar 25		6			125 ug LHRHa (China) implantation (4 3*, 1 3**)	-	-	-
12,		8	Jun 18					250 ug LHRHa (China)	Jun 21	499,500	1.175
13:		8	Mar 31 Jun 18	, Apr	23,	May 2	11	implantation* 200 IU HCG		177,200	4.4/3
10: 10:		9 9-11									
19:	IV	8-10	May 24			•		1.0 ml Hoe 766	-	-	-
58:	IV	8-10	May 24					(500 ug LHRHa) 300 IV HCG			
2 🕂 1	III	8-10	May 24					1.0 ml Hoe 766	May 25	649,600	1.175
32 :	III	8-10	May 24					(500 ug LHRHa) 300 IU HCG			
4 🖞 🖬	III	8-10	May 24					0.5 ml Hoe 766 (250 ug LHRHa)	May 25	399,600	1.151

## Table 🔲 Record on spawning of milkfish in TML in 1986

Implantation of 200 ug LHRHa

\*\* Implantation of 100 ug LHRHa

- 41'

Table 📗

Record on inspection and treatment of milkfish in pond III

(paddle wheel aerator)

Fish	May 2		Jun 20							
tag	BW (kg)	FL (cm)	condition	BW (kg)	FL (cm)	condition				
PC	2.80	54.0	-	2.48	54.0	-				
TC	4.00	58.0	0.445	3.64	58.0	-				
BC	4.10	58.5	-	3.68	59.4	injured				
TW	5.80	69.0	0.713 🔊	5.00	69.0	-				
BW	2.50	52.7	-	2.40	53.0	-				
DPC	3.20	58.3	+++ 5	2.92	58.0	-				
DTC	3.45	56.2	0.685	2.95	5ó.O	-				
DBC	3.45	58.5	-	3.12	58.8	-				
DTW	3.50	58.0	-	2.98	58.5	-				
DBW	3.40	55.5	0.840	2.66	54.8	-				
АРС	3.98	61.5	-	3.42	61.0	-				
ATC	4.18	61.0	-	3.88	61.6	-				
ABC	3.74	59.3	0.625	3.30	58.5	-				
ATW	2.64	50.5	-	2.36	50.7	-				
ABW	2.86	52.5	+++	2.66	52.5 .	-				
ADPC	2.70	53.0	-	2.50	53.5	<b>.</b>				
ADTC	2.86	53.9	0.738	2.54	53.3	-				
ADBC	4.68	63.5	-	4.16	63.4	-				
ADTW	3.70	58.5	-	3.40	58.6	-				
ADBW	2.88	53.5	-	2.54	53.0	-				
bc	2.88	54.5	-	2.52	54.3	-				
ADC	3.00	55.7	0.728 \bullet	2.68	55.0	-				
ADPTC	4.30	62.5	-	4.04	62.7	-				
UDPBC	4.24	59.0	atresia	3.92	59.2	-				
DPTW	2.96	52.5 ·	0.830 🗨	2.54	52.7	-				
DPBW	3.80	60.1	_	3.60	59.5	_				

S Fish were moved to spawning pond for spawning.

Fish spawned.

• milt appear yellowish in colour

milt: + Scarce; ++ Madium; +++ Abundant

Fish	May 2	3		Jun	20	
tag	BW	FL		BW	FL	
	(kg)	( ca)	condition	(kg)	( 📼 )	condition
PC	2.18	49.5	-	2.22	49.8	<u> </u>
TC	3.50	58.0	-	3.46	57.6	+
8C	2.90	54.5	-	2.94	54.5	+++
TW	4.76	62.0	0.792 5	4.24	60.6	-
SW	3.56	57.0	-	3.60	58.0	-
DPC	2.90	54.6	-	2.76	55.0	-
DTC	3.06	55.0	++ S)	2.76	54.3	-
DBC	4.30	63.5	-	4.38	64.0	***
DTW	2.40	50.5	-	2.36	50.7	-
DBW	3.10	55.0	+ \$	3.01	54.5	-
APC	3.20	55.9	0.300	3.00	5á.O	-
ATC	3.04	55.0	-	2.96	55.5	-
ABC	4.20	60.5	previt.	4.12	60.3	-
atw	2.80	54.9	0.450	2.72	54.5	-
ABW	4.10	60.0	· <b>-</b>	4.02	60.5	-
ADPC	2.85	54.5	atresia	2.84	54.5	-
ADTC-	3.22	56.5	-	3.06	57.1	0.449
ADBC	3.40	58.0	+++ 5	Dead (		= 84.15 g)
ADTW	2.60	52.5	+•	2.45	52.4	-
ADBW	2.92	55.0	++ \$	2.30	54.5	-
bC .	2.90	54.5	atresia	2.78	54.5	-
ADC	4.00	58.5	-	3.90	58.0	+
ADPTC	3.13	54.3	<b>4</b>	2.98	54.0	-
Deac	2.22	51.5	+	2.13	52.5	-
Detw	2.50	52.0	-	2.38	52.0	-
WEGO	2.70	53.0	+ 5	2.50	52.4	_

Table V Record on inspection and treatment of milkfish in pond IV (air blower)

S Fish were moved to spawning pond for spawning.

• milt appear yellowish in colour

milt: + scarce; ++ medium; +++ abundant.

previt.: previtellogenesis

Fish tag	Hay 19 milt (ml)	sperm	Jun 1 milt (m1)	sperm act.	Jul 1 milt (ml)	lO sperm act.	milt	sperm act.	Sep 3 milt (ml)	sperm		sperm act.	Oct 29 milt (ml)	Nov 26- Mar 18, 1986 milt(ml)	λpr 24* milt (ml)	Jun 10** milt (ml)
Expt.	fish															
тс	0.8	F F	-		1.2	++ + +	0.8	++++	0.01	++	-		-	-	abundant	abundant
BC	0.59	* * * *	1.8	++++	0.1	++++	0.2	++++	<b>-</b>		-		-	-	-	-
B₩	0.61	+++	-		scard	e	-				-		-	. <del></del>	-	_
DBC	-		1.4	+++	scard	e	scarc	e	-		-		scarce	-	-	_
DBW	-		-		-		-		-		-		-	_	-	_
Contre	51.															
тс	-		0.8	+++	-		-		0.04	++++	-		-	_	_	_
BC	-		0.2	++++	0.05	+++	-		0.81		0.1	++++	_	_	-	_
T₩	-		0.8	++++	0.25	+++	0.62	++++	0.95		0.15	+++	_	_	_	-
BW	-		0.6	-	-		scarc		-		-		-	-	-	-

Table V Record of milt collection from milkfish in the methyltestosterone feed experiment (1985-1986)

\* Fish were moved to new ponds on Har 21, 1986.

\*\* Fish were moved to new ponds on Hay 11, 1986.

Table M Result for inspection of the 1985 hormone implantation experimental fish

R10 (age: 10)

Fish tag	Apr 2 FL (cm)	27*	Hay 27	Jun 26	Jul 24	Λug 21	Sep 18	0ct 16	Nov 13	Dec 11, 1985 Feb 5, 1986	Mar 31** BW FL (kg)_(cm)	λpr 24▼	May 23	Jun 18
BC	68.5	-	÷	÷	-	+	~	<b>*</b> •	++•		5.51 68.9	_		
TW	64.5	-	-	-	+	Ŧ	-	÷	÷	-	5.40 68.5	-	-	_
DTW	59.5	-	-	-	+++	F	-	-	÷	-	4.98 63.1	-	-	-
DBW	58.5	-	-	+	***	۲.	-	<b>+</b> *	+	-	4.20 61.0	-	_	-
TC	64.0	-	-	-	-	-	-	-	-	-	5.50 65.4	_	-	_
DBC	53.5	-	-	-	F F	ŧ	-	_	-	-	Dead ( ô, G	w - 10	), 168	<b>"</b> )
DTC	51.5	-	Dead	( 8.	GW =	0.98	g)					-		37
BW	60.5	-			G₩ ≕									
R9 (a	ge: 5)													
DBC	52.0		+	+	-	-	-	-	-	-				
TW	49.0	-	+	-	-	+	-	F	-	-				
DTW	48.0	-	-	-	_	_	-	_	-	-				
DBW	49.0	-	+	-		-	-	-	-	-				
BW	48.0	-	-	-	-	-	-	-	_	-				
DTC	48.0	-	-	-	-	-	-	_	_	_				
IC	52.0	-	_	-	-	-	-	-	_	-				
вс	52.0			<b>D</b> = = 2		GW =								

\* Fish were implanted with 180 ug LHRHa + 250 ug liquid testosterone.

\*\* Fish were moved to new ponds.

Ten-year-old fish were implanted with 200 ug LHRHa + 250 ug  $17 \ll$  - HT.

- ▼ Fish were implanted with 200 ug LHRHa.
- milt appear yellowish in colour
  - milt: + Scarce; ++ Medium; +++ Abundant

.

Fish tag	BW (kg)	Mar 26 FL (cm)	condition*	Apr 23	May 21	Jun 18
	(\\\y)	( Cm)		condition*	condition*.	condition
Expt.	fish		•			
PC	1.18	42.3	-	-	_	-
TC	1.25	42.8	-	-	-	-
BC	0.91	39.2	-	-	-	-
TW	1.02	38.0	-		-	-
BW	0.95	39.7	-	-	-	-
VbC	1.14	38.1	-	-	-	-
λtc	0.97	37.5	-	-	-	-
νвс	0.98	38.5	-	-	-	-
λtw	1.02	39.0	-	-	-	-
VBM	0.98	39.0	-	-	-	-
Contro	51					
DPC	1.14	38.1	-	-	-	-
DTC	0.72	35.8	-	-	-	-
DBC	1.02	39.3	-	-	-	-
DTW	0.84	38.9	-	-	-	-
DBW	1.01	39.0	-	-	-	-
олтс	0.74	35.8	-	-	-	_

Table WH Record on inspection and treatment of milkfish in pond R8

5

(4 years old) ( $\lambda$ ge - Implantation Expt.)

\* Experimental fish were implanted with pellets containing 200 ug LHRHa.

Control fish were implanted with cholesterol pellets.

Fish tag	BW	Mar FL	26	Apr 23	May 21		Jun	18
Lag	(kg)	(cm)	condition*	condition*	condition*	B₩ (mg)	FL (cm)	condition
Expt. fi	lsh							
PC	1.94	47.3	-	-	-	2.18	49.0	_
тс	3.32	57.8	(Dead:♂, GW = 0.08 g)	replaced	-	2.30	51.5	-
BC	3.23	57.4	-	<del>-</del> .	_	3.70	60.9	_
TW	1.98	48.0	-	-	+++	2.06	50.0	-
BM	2.72	51.8	previt.	-	-	2.88	52.5	-
Vac	1.80	47.8	-	_	-	2.02	50.5	+•
ATC	2.30	50.3	-	-	-	2.60	52.5	_
VBC	2.18	50.5	-	-	-	2.50	52.0	+
NTW	1.80	46.5	-	_	-	1.90	48.0	+
NBW	2.15	50.0	-	-	-	2.42	51.0	+
Control								
DFC	2.38	51.8	-	-	-	2.52	53.3	-
DTC	2.24	50.7	-	-	-	2.42	50.5	_
DBC	1.88	47.6	-	-	-	2.10	49.5	-
DTW	2.93	54.0	-	_	-	3.14	55.5	-
DBW	1.94	48.2	-	-	-	1.90	49.0	_
DATC	2.14	49.0	-	~	-	2.16	51.3	-

Table X Record on inspection and treatment of milkfish in pond R3 (5 years old) (Age -Implantation Expt.)

\* Experimental fish were implanted with pellets containing 200 ug LiRHa.

Control fish were implanted with cholesterol pellets.

• Milt appear yellowish in colour.

Hilt: + Scarce: ++ Medium; +++ Abundant

Previt.: previtellogenesis

1 3

.

Fish Tag	BW	Mar 26 FL	5	Apr 23	May 21		Jun	18
	(kg)	( cm )	condition*	condition*	condition*	BW (kg)	FL (cm)	condition
Sxpt.	fish							
с	1.58	44.4	-	_	-	1.92	47.5	_
.C	1.76	46.8	-	_	<b>+</b> •	2.10	49.8	_
C	1.58	44.7	-	-	-	1.80	48.1	-
W	2.51	56.3	<b>-</b> ·	-	-	2.80	57.0	0.529
W.	1.68	46.6	-	-	-	2.00	49.5	-
PC	2.12	50.0	-	-	-	2.26	52.0	-
TC	2.22	51.5	-	-	+•	2.66	55.5	-
BC	2.87	52.8	-	+	.+•	3.10	55.5	+
TW	2.02	50.0	-	-	-	2.42	53.0	* ++
BW	2.51	52.3	-	+	-	2.95	54.5	+
ontro	<b>51</b>							
PC	2.70	54.2	-	-	-	Hissin		
TC	3.15	55.5	-	-	++	3.68	58.0	-
BC	2.73	53.7	-	-	-	3.38	57.5	_
TW	1.76	47.8	-	-	-	2.00	49.5	_
BW	1.81	46.6	-	-	+	2.06	50.7	+•
λtc	2.18	50.0	-	-	_	2.60	53.5	-

Table X Record on inspection and treatment of milkfish in pond R4 (6 years old)

(Age - Implantation Expt.)

\* Experimental fish were implanted with pellets containing 200 ug LIRHa. Control fish were implanted with choiesterol pellets.

• Milt appear yellowish in colour.

```
Milt: + Scarce; ++ Medium; +++ Abundant
```

egg: diameter in mm.



TableXI Record on inspection and treatment of milkfish in pond R9 (7 years old)

(Age - Implantation Expt.)

Fish tag	BW	Mar FL	27	λpr 24	May 21			18
	(kg)	(cm)	condition*	condition*	condition*	Bw (kg)	FL (cm)	condition
Expt.	fish					· · · · · · · ·		
PC	2.91	53.4	-	-	-	3.38	57.0	-
TC	4.16	58.6	-	-	-	3.32	58.5	-
BC	3.02	56.5	-	-	+	3.10	58.0	+
TW	3.78	60.7	-	-	+++	3.50	58.8	+
BW	4.20	60.7	previt.	-	atresia	4.00	61.0	-
VLC	2.55	51.7	-	-	+•	2.78	54.0	-
VIC	2.58	52.5	-	-	0.357	2.60	53.0	-
VBC	2.89	55.4	-	+	-	2.54	54.5	-
λtw	3.19	56.8	-	-	-	3.42	60.4	-
νbm	3.21	55.4	-	-	-	3.22	57.0	-
Contro	51							•
DPC	2.28	50.8	previt.	-	-	2.40	54.5	_ ·
DTC	3.42	58.4	-	-	-	4.32	64.5	_
DBC	3.06	56.5	-	-	<b>+</b>	3.05	56.5	-
DTW	3.31	55.0	-	-		3.42	57.5	-
DBW	3.26	57.0	-	-	<b>-</b> '	3.08	56.5	_
DATC	2.77	53.0	-	-	-	2.84	52.8	_

\* Experimental fish were implanted with pellets containing 200 ug LHRHa. Control fish were implanted with cholesterol pellets.

• Milt appear yellowish in colour.

Milt: + Scarce; ++ Medium; +++ Abundant

egg: diameter in mm; previt.: previtogenesis.

Fish tag	BW	Har Fl.	26	λpr 23	Hay 12	May 21		Jun	18
	(kg)	(cm)	condition*	condition*	condition	condition*	BW (kg)	FL (cm)	condition
Expt.	fish								
PC	3.40	56.6	-	+++	+	-	3.10	56.5	•
тс	4.00	57.8	-	-	-	-	3,80	58.5	-
BC	4.22	57.9	- :	* * *	<b>;</b> •	++ 30	3.68	58.0	+
TW	3.00	56.0	-	-	-	-	3,22	57.3	
BW	3.13	54.0	-	-	-	-	2.74	54.0	
NPC	3.71	58.8	-		+++	•	3.62	59.5	-
NTC	3.05	54.0	-	-	atresia	-	2.85	55.0	<u> </u>
NBC	4.31	59.8	-	0.782 🥱	-	-	3.34	58.8	-
ΛTW	3.22	55.0	~	***	<b>+</b> •	+•	3.14	55.5	+++ 5
NBM	4.33	57.9	-	-	+•	-	3.75	58.5	-
DVBM			-	<del>-</del> .	-	-	2.90	54.5	-
Contro	<b>51</b>								
DFC	4.00	59.5	-	0.681	-	0.651	3.78	59.5	-
DTC	3.82	57.5	-	0.531	-	-	3.18	56.0	-
DBC	2.68	53.0	0.355	-	-	-	2.52	53.0	-
DTW	3.62	56.4	-	-	0.330	-	3.44	58.3	-
98W	3.92	58.8	0.590	-	-	-	3.68	59.0	0.785 🖝
DATC	3.68	57.4	-	-	-	<b>+•</b>			= 12.009g)

, E

Table X Record on inspection and treatment of milkfish in pond R5 (8 years old)

(Age - Implantation Expt.)

\* Experimental fish were implanted with pellets containing 200 ug LHRHa.

Control fish were implanted with cholesterol pellets.

- Hilt appear yellowish in colour
- S Fish were moved to spawning pond for spawning.

## S Fish spawned.

Milt: + Scarce; ++ Medium; +++ Abundant

egg: diameter in mm.

Fish tag	BW	Mar FL	27	λpr 24	May 21	Jun 18		
	(kg)	(cm)	condition*	condition*	condition*	BW (kg)	FL ( cm )	. condition
Expt.	fish				*****			
PC	3.41	55.8	-	+++ <b>(</b>	-	3.36	56.5	+++ (5)
ГC	4.47	62.7	-	-	0.674	4.40	63.0	-
BC	2.97	55.0	-	-	+	3.58	59.0	0-847 ●
rw	2.94	53.0	-	- ·	+++ (S)	3.42	55.5	-
3W	3.25	57.0	0.390	-	0.742 ●	3.10	57.5	-
VPC	4.19	61.7	-	+++ G	-	4.50	63.0	++ 🕲
\TC	5.07	63.9	previt.	-	-	5.45	66.0	~
NBC	3.25	54.0	+	+++	+++ 🕲	3.25	54.7	-
\TW	2.67	52.8	-	-	-	2.90	55.0	-
NBM	3.30	54.6	-	-	-	3.12		+++ 3
ontro	<b>51</b>							
PC	4.19	63.6	-	+++ <b>S</b>	-	Hissing		
TC	3.42	57.7	-	-	-	3.58		-
BC	4.10	60.0	-	+++	+++ (g)	4.20	61.0	++ 🕲
TW	4.10	59.3	-	·_	-	4.40	61.5	-
BW	3.02	54.6	-	-	-	3.22	57.5	_
ATC	3.32	58.8	-	-	+	3.94	60.0	-

Table XM Record on inspection and treatment of milkfish in pond R10 (8-10 years old)

(Age - Implantation Expt.)

\* Experimental fish were implanted with pellets containing 200 ug LHRHa. Control fish were implanted with cholesterol pellets.

S Fish were moved to spawning pond for spawning

S Fish spawned

Hilt: + Scarce; ++ Medium; +++ Abundant.

egg: diameter in mm; previt.: previtogenesis

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Age of fish	Mar 26* male female		Apr 23* male female		May 21* male female		Jun 18 male female	
Expt. Fish**								
4	0	0	0	0	0	0	0	0
5	0	1	0	0	1	0	4	0
6	0	0	2	0	3	0	3	1
7	0	1	1	0	3	1	2	0
8	0	0	3	1	3	0	3	0
8 - 10	1	2	3	0	3	2	3	1
Control**								
4	0	0	0	0	0	0	0	0
5	0	0	0	0	1	0	0	0
6	0	0	0	0	2	0	1	0
7	0	1	0	0	1	0	0	0
8	0	2	0	2	1	1	0	1
8 - 10	0	0	2	0	2	0	1	0

Table XIII Numbers of mature milkfish identified in the Age-Implantation Experiment at TML in 1986.

\* Experimental fish were implanted with pellets containing 200 ug LHRHa. Control fish were implanted with cholesterol pellets.

\*\* Each age group has 10 experimental fish and 6 control.